Claims

1. A method for stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells, comprising contacting, *in vitro* or *ex vivo*, the surface of the cells with both a lentiviral vector and at least one molecule which binds the cell surface, and culturing the cells in a ventilated vessel comprising two or more layers under conditions conducive to growth and/or proliferation, wherein the vessel is suitable for culturing at least about 100 million cells.

2. A method for stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells, comprising contacting, *in vitro* or *ex vivo*, the surface of the cells with both a lentiviral vector and at least one molecule which binds the cell surface, and culturing the cells in a ventilated vessel comprising two or more layers under conditions conducive to growth and/or proliferation, wherein the vessel is suitable for culturing at least about 100 million cells; and

wherein the cells are transduced with the lentiviral lentiviral vector at a multiplicity of infection (MOI) such that the copies of lentiviral lentiviral vector per transduced cell is from about 0.5 to about 10; and

wherein contacting the cells with a lentiviral vector is for about 24 hours and is optionally repeated at least once.

3. A method for stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells, comprising contacting, *in vitro* or *ex vivo*, the surface of the cells with both a lentiviral vector and at least one molecule which binds the cell surface, and culturing the cells in a ventilated vessel comprising two or more layers under conditions conducive to growth and/or proliferation, wherein the vessel is suitable for culturing at least about 100 million cell; and

wherein at least about 50% of the cells are stably transduced after about seven to ten days, or at about 14 days; and optionally at least 50% of the cells remain stably transduced after about 14 days; or

wherein at least about 75% of the cells are stably transduced after about seven to ten days, or at about 14 days, and optionally at least 75% of the cells remain stably transduced after about 14 days; or

wherein greater than 80%, 85%, 89%, 90%, 91%, 92%, 93%, 94% or 95% of the cells are stably transduced after about 14 days; or

wherein the cells are transduced with the lentiviral vector at a multiplicity of infection (MOI) of from about 2 to about 50, or from about 10 to about 30, or from at 10, or at about 20, or at about 30, or at about 40, or at about 50, or from about 1 to about 400, or less than 500; or

wherein the cells are transduced with the lentiviral vector at a multiplicity of infection (MOI) such that the copies of lentiviral vector per transduced cell is from about 1 to about 100; or

wherein the cells are transduced with the lentiviral vector at a multiplicity of infection (MOI) such that the copies of lentiviral vector per transduced cell is from about 0.5 to about 10; or

wherein contacting the cells with a lentiviral vector is for about 24 hours and is optionally repeated at least once; and

wherein the cell surface molecule does not induce apoptosis and the cell surface binding molecule results in the cell being more receptive to transduction by a viral lentiviral vector.

4. The method of claim 1, wherein the primary cells are isolated or derived from a subject.

5. The method of claim 4, wherein the primary cells are isolated by one or more of the following procedures:

(a) by apheresis of a subject's blood; or

(b) from bone marrow from a subject's bone; or

(c) by apheresis of an allogeneic subject's blood; or

(d) from bone marrow from an allogeneic subject's bone.

6. The method of claim 4, wherein the subject is infected with a human immunodeficiency virus (HIV), wherein optionally the HIV is HIV-1 or HIV-2.

7. The method of claim 4, wherein the subject has cancer, wherein optionally the cancer is breast cancer.

8. The method of claim 4, wherein the subject is a human or an animal.

9. The method of claim 1, wherein the primary cells are enriched prior to contact with the lentiviral vector or cell surface binding molecule by passing the cells over a gradient density buffer and/or by immuno-purification over a magnetic field.

10. The method of claim 1, wherein the contacting of the primary cells:

(a) with the lentiviral vector occurs before contacting the cells with at least one cell surface binding molecule; or

(b) with the lentiviral vector occurs simultaneously with contacting the cells with at least one cell surface binding molecule; or

(c) with the lentiviral vector occurs after contacting the cells with at least one cell surface binding molecule; or

(d) with the lentiviral vector occurs more than once; or

(e) with the lentiviral vector occurs continuously, after the simultaneous contacting of the cells with the lentiviral vector and the at least one cell surface binding molecule; or

(f) with cell surface binding molecule occurs continuously, after the simultaneous contacting of the cells with the lentiviral vector and the at least one cell surface binding molecule; or

(g) with the lentiviral vector and the at least one cell surface binding molecule occurs continuously, after the initial simultaneous contact of the cells with the lentivirus vector and the at least one cell surface binding molecule; or

(h) wherein any of (a) through (g) occurs at least once over a time period of about 24-36 hours.

11. The method of claim 1, wherein the primary cells are pre-stimulated with at least one cell surface binding molecule, and optionally the cells are pre-stimulated with the at least one cell surface binding molecule within about twenty four (24) hours prior to simultaneously contacting the cells with the lentiviral vector and the at least one cell surface binding molecule, or and optionally the cells are pre-stimulated with the at least one cell surface binding molecule within about 12 to 96 hours prior to simultaneously contacting the cells with the lentiviral vector and the at least one cell surface binding molecule.

12. The method of claim 1, wherein the lentiviral vector comprises at least one cis-acting nucleotide sequence derived from the gag, pol, env, vif, vpr, vpu, tat or rev genes,

and optionally, wherein the sequence is not expressed or is a fragment or a mutant of the gag, pol, env, vif, vpr, vpu, tat or rev genes.

13. The method of claim 1, wherein the lentiviral vector is:

(a) pseudotyped and optionally wherein the pseudotyped vector contains the vesicular stomatitis virus G envelope protein; or

(b) pseudotyped, and wherein the pseudotyping comprises co-transfecting or co-infecting a packaging cell with both the lentiviral vector genetic material and genetic material encoding at least one envelope protein of another virus or a cell surface molecule; or

(c) pseudotyped with a *Rhabdovirus*, and optionally wherein the *Rhabdovirus* is a Vesicular Stomatitis Virus envelope G (VSV-G) protein.

14. The method of claims 1, wherein the primary cell is a lymphocyte, a precursor of a lymphocyte, a CD4 positive cell, a hematopoietic stem cell of a CD4 positive cell, a CD8 positive cell, a hematopoietic stem cell of a CD8 positive cell, a CD34 positive cell, a hematopoietic stem cell of a CD34 positive cell, a dendritic cell, a cell capable of differentiating into a dendritic cell, a human primary cell of the hematopoietic system and/or a human hematopoietic stem cell, a precursor of a human hematopoietic stem cell, an astrocyte, a skin fibroblast, a epithelial cell, a neuron, a dendritic cell, a leukocyte, a cell associated with the immune response, a vascular endothelial cell, a tumor cell, a tumor vascular endothelial cell, a liver cell, a lung cell, a bone marrow cell, an antigen presenting cell, a stromal cell, an adipocyte, a muscle cell, a pancreatic cell, a kidney cell, an ovum, a spermatocyte, a cell that contributes to the germ line, an embryonic pluripotential stem cell or a progenitor cell, a blood cell, a non-nucleated cell, a platelet cell, or an erythrocyte, or a derivative thereof.

15. The method of claim 1, wherein the at least one cell surface binding molecule:

(a) comprises a polypeptide, a lipid, a nucleic acid, a carbohydrate or an ion; or

(b) comprises an antibody, an antigen binding fragment, a ligand, or a cell surface molecule; or

(c) comprises FLT-3 ligand, TPO ligand, or Kit ligand, or a polypeptide or other binding molecule that is a cell surface binding analog of FLT-3 ligand, TPO ligand, or Kit ligand; or

(d) comprises CD34, CD3 ligand, CD28 ligand, CD25 ligand, CD71 ligand, or CD69 ligand, or a polypeptide or other binding molecule that has the same cell surface binding specificity of CD34, CD3, CD25, CD28, CD69 or CD71 ligand; or

(e) comprises a composition comprising GM-CSF, IL-4, and TNF-alpha; GM-CSF and interferon-alpha; or a polypeptide or other binding molecule that is a cell surface binding analog of GM-CSF, IL-4, and TNF-alpha; GM-CSF or interferon-alpha; or

(f) comprises a CD3 antibody or cell surface binding fragment thereof, a CD28 antibody or cell surface binding fragment thereof, a combination of the antibody and cell surface binding fragment thereof, and a binding molecule that has the same cell surface binding specificities as the antibody; or

(g) comprises a combination of CD3 and CD28 antibodies immobilized on a bead or a surface, wherein optionally the bead or surface comprises coated beads; or

(h) comprises two or more cell surface binding molecules selected from any of (a) through (g); or

(i) comprises another molecule that is used to increase or reinforce the ability of the molecule to bind to the surface of the cell; or

(j) is complexed with another molecule; or

(k) is found on the primary cell's surface and binds to the surface of another cell.

16. The method of claim 1, wherein the conditions comprise:

(a) further incubation with a cell surface binding molecule or a cytokine; or

(b) further incubation with interleukin-2; or

(c) culturing the cells for about seven days; or

(d) culturing the cells for about 14 days.

17. The method of claim 1, wherein the lentiviral vector is:

(a) derived from a human immunodeficiency virus (HIV); or

(b) derived from HIV-1, HIV-2, or a combination thereof; or

(c) a chimeric vector comprising HIV sequences, wherein optionally the HIV sequences comprise HIV-1 and HIV-2 sequences; or

(d) VRX496 or a derivative thereof.

18. The method of claim 1, wherein said contacting occurs *ex vivo* in a mixed or pure cell culture, a tissue or an organ system.

19. A method to introduce a genetic material into a cell comprising *ex vivo* introduction of the cell transduced by the method of claim 1 into a living subject, a tissue, an organ, a blastocyst or an embryonic stem cell.

20. Use of a primary cell of the hematopoietic system or hematopoietic stem cell transduced by the method of any one of claims 1 to 18 for the preparation of a pharmaceutical composition.

21. Use of a primary cell of the hematopoietic system or hematopoietic stem cell transduced by the method any one of claims 1 to 18, for the preparation of a pharmaceutical composition for the treatment or prevention of a viral infection in a subject.

22. Use of a primary cell of the hematopoietic system or hematopoietic stem cell transduced by the method any one of claims 1 to 18, for the preparation of a pharmaceutical composition for the treatment or prevention of an HIV infection in a subject.

23. Use of a primary cell of the hematopoietic system or hematopoietic stem cell transduced by the method any one of claims 1 to 18, for the preparation of a pharmaceutical composition for the treatment or prevention of cancer.

24. The use of claim 23, wherein the cancer is breast cancer or a cancer of the endothelial cells.

25. A pharmaceutical composition for gene therapy to treat or prevent an abnormality caused by a genetic defect, or to treat, diagnose, alleviate or prevent a tumor or a cancer, produced by the method of any one of claims 1 to 18, and optionally wherein the abnormality caused by a genetic defect or tumor or cancer is a breast cancer tumor.

26. A pharmaceutical composition for gene therapy to treat or prevent an abnormality caused by an infection, produced by the method of any one of claims 1 to 18.

27. The pharmaceutical composition of claim 26, wherein the infection is a viral infection, and optionally wherein the viral infection is a human immunodeficiency virus (HIV) infection.

28. The pharmaceutical composition of claim 25, wherein the pharmaceutical composition is formulated for use *ex vivo*.

29. The pharmaceutical composition of claim 26, wherein the pharmaceutical composition is formulated for use *ex vivo*.

30. A method for stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells, comprising contacting, *in vitro* or *ex vivo*, the surface of the cells with both a lentiviral vector and at least one molecule which binds the cell surface, and culturing the cells in a ventilated vessel comprising two or more layers under conditions conducive to growth and/or proliferation, wherein the vessel is suitable for culturing at least about 100 million cells, and wherein the contacting of the primary cells with the cell surface molecule makes the cells more receptive to transduction by the lentiviral vector.

31. The method of claim 30, wherein the presence of the cell surface molecule results in:

(a) the cell's chromatin being more receptive to DNA integration; or

(b) integration of the lentiviral vector into a cellular site favorable for expression of a gene from the lentiviral vector; or

(c) more efficient entry of a nucleic acid containing capsid into the cytoplasm of the cells; or

(d) more efficient entry of the virus across a cell membrane or across an internal membranous structure of the cells; or

(e) the primary cells being more permissive for nuclear import of the genetic material contained in the viral vector.

32. The method of claim 1 or claim 31, wherein the cell surface binding molecule, antibody, antigen binding fragment, ligand or cell surface molecule comprises: anti-CD3 or anti-CD28 antibodies which bind the cells and make them more receptive to vector

transduction; antibodies or ligands for the FLT-3 ligand, TPO, and Kit ligand receptors, which bind the cells and make them more receptive to vector transduction; antibodies or ligands for GM-CSF and IL-4 receptors, which bind dendritic cells or their precursors, monocytes, CD34 positive stem cells, or their differentiated progenitor cells on the dendritic cell lineage, and make them more receptive to vector transduction; a polypeptide, nucleic acid, carbohydrate, lipid or ion, or a polypeptide, nucleic acid, carbohydrate, lipid or ion complexed with another substance that binds CD 1 a, CD 1 b, CD 1 c, CD 1 d, CD2, CD3γ, CD3δ, CDε, CD4, CD5, CD6, CD7, CD8 α, CD8β, CD9, CD10, CDlla, CD11b, CDllc, CDw12, CD13, CD14, CD15, CD15s, CD16a, CD16b, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42a, CD42b, CD42c, CD42d, CD43, CD44, CD45, CD45R, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CDw60, CD61, CD62E, CD62L, CD62P, CD63, CD64, CD65, CD66a, CD66b, CD66c, CD66d, CD66e, CD66f, CD67, CD68, CD69, CDw70, CD71, CD72, CD73, CD74, CDw75, CDw76, CD77, CD79cc, CD79(3, CD80, CD81, CD82, CD83, CD84, CD85, CD86, CD87, CD88, CD89, CD90, CD91, CDw92, CD93, CD94, CD95, CD96, CD97, CD98, CD99, CD100, CD101, CD102, CD103, CD 104, CD 105, CD 106, CD 107a, CD 107b, CDwl08, CDwl 09, CD 114, CD 115, CD 116, CD117, CD118, CD119, CD120a; CD120b, CD121a, CD121b, CD122, CD123, CDw124, CD125, CD126, CDwl27, CDw128a, CDwl28b, CDwl30, CDw131, CD132, CD133, CD134, CD135, CD136, CDw137, CD138, CD139, CD140a, CD140b, CD141, CD142, CD143, CD144, CDw145, CD146, CD147, CD148, CDw149, CD150, CD151, CD152, CD153, CD154, CD155, CD156, CD157, CD158a, CD158b, CD161, CD162, CD163, CD164, CD165, CD166 or TCRζ on the cells and makes them more receptive to vector transduction.

33. A method for stable transduction of a primary cell of the hematopoietic system and/or a hematopoietic stem cell isolated from an HIV-infected subject, comprising the steps of:

(a) isolating from the HIV-infected subject primary cells of the hematopoietic system cells or hematopoietic stem cells;

(b) optionally, pre-stimulating the primary cells or hematopoietic stem cells with at least one cell surface binding molecule;

(c) contacting simultaneously *in vitro* or *ex vivo* the hematopoietic system cells or hematopoietic stem cells with a lentiviral vector and at least one cell surface binding molecule; and

(d) culturing the cells in a ventilated vessel comprising two or more layers under conditions conducive to growth and/or proliferation, wherein the vessel is suitable for culturing at least about 100 million cells.

34. A system comprising:

(a) a ventilated vessel comprising two or more layers; and

(b) isolated non-adherent primary cells of the hematopoietic system and/or hematopoietic stem cells.

35. The system of claims 34, wherein the primary cell is a lymphocyte, a precursor of a lymphocyte, a CD4 positive cell, a hematopoietic stem cell of a CD4 positive cell, a CD8 positive cell, a hematopoietic stem cell of a CD8 positive cell, a CD34 positive cell, a hematopoietic stem cell of a CD34 positive cell, a dendritic cell, a cell capable of differentiating into a dendritic cell, a human primary cell of the hematopoietic system and/or a human hematopoietic stem cell, a precursor of a human hematopoietic stem cell, an astrocyte, a skin fibroblast, a epithelial cell, a neuron, a dendritic cell, a leukocyte, a cell associated with the immune response, a vascular endothelial cell, a tumor cell, a tumor vascular endothelial cell, a liver cell, a lung cell, a bone marrow cell, an antigen presenting cell, a stromal cell, an adipocyte, a muscle cell, a pancreatic cell, a kidney cell, an ovum, a spermatocyte, a cell that contributes to the germ line, an embryonic pluripotential stem cell or a progenitor cell, a blood cell, a non-nucleated cell, a platelet cell, or an erythrocyte, or a derivative thereof.

36. The system of claim 34, wherein the multilayer vessel is rectangular in shape, square in shape, or rectangular in shape with a curved edge, or square in shape with a curved edge.

37. The method of claim 34, wherein the vessel is suitable for culturing at least about 100 million cells.